

RESEARCH PAPER

# Calcium partitioning and allocation and blossom-end rot development in tomato plants in response to whole-plant and fruit-specific abscisic acid treatments

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## Abstract

The mechanisms regulating  $\text{Ca}^{2+}$  partitioning and allocation in plants and fruit remain poorly understood. The objectives of this study were to determine  $\text{Ca}^{2+}$  partitioning and allocation in tomato plants and fruit in response to whole-plant and fruit-specific abscisic acid (ABA) treatments, as well as to analyse the effect of changes in  $\text{Ca}^{2+}$  partitioning and allocation on fruit susceptibility to the  $\text{Ca}^{2+}$  deficiency disorder blossom-end rot (BER) under water stress conditions. Tomato plants of the cultivar Ace 55 (Vf) were grown in a greenhouse and exposed to low  $\text{Ca}^{2+}$  conditions during fruit growth and development. Starting 1 day after pollination (DAP), the following treatments were initiated: (i) whole plants were sprayed weekly with deionized water (control) or (ii) with  $500 \text{ mg l}^{-1}$  ABA; or fruit on each plant were dipped weekly (iii) in deionized water (control) or (iv) in  $500 \text{ mg l}^{-1}$  ABA. At 15 DAP, BER was completely prevented by whole-plant or fruit-specific ABA treatments, whereas plants or fruit treated with water had 16–19% BER incidence. At 30 DAP, BER was prevented by the whole-plant ABA treatment, whereas fruit dipped in ABA had a 16% and water-treated plants or fruit had a 36–40% incidence of BER. The results showed that spraying the whole plant with ABA increases xylem sap flow and  $\text{Ca}^{2+}$  movement into the fruit, resulting in higher fruit tissue and water-soluble apoplastic  $\text{Ca}^{2+}$  concentrations that prevent BER development. Although fruit-specific ABA treatment had no effect on xylem sap flow rates or  $\text{Ca}^{2+}$  movement into the fruit, it increased fruit tissue water-soluble apoplastic  $\text{Ca}^{2+}$  concentrations and reduced fruit susceptibility to BER to a lesser extent.

**Key words:** ABA, apoplast, blossom-end rot, leakage, membrane, xylem sap.

## Introduction

Calcium ( $\text{Ca}^{2+}$ ) is an essential plant nutrient required for proper plasma membrane function, in storage organelles to counterbalance anionic charges, in the cytosol for cellular signalling responses, and in the apoplast for cell wall structure (White and Broadley, 2003; Taylor and Locascio, 2004; Ho and White, 2005).  $\text{Ca}^{2+}$  deficiency disorders in fruit have

been attributed to lower total tissue  $\text{Ca}^{2+}$  content, as well as abnormal regulation of cellular  $\text{Ca}^{2+}$  partitioning and distribution (Ho and White, 2005; Park *et al.*, 2005; De Freitas *et al.*, 2011a). The symptoms of  $\text{Ca}^{2+}$  deficiency disorders in fruit start with cell plasmolysis and the water-soaked appearance of blossom-end tissues that eventually becomes

Abbreviations: ABA, abscisic acid; BER, blossom-end rot; C, carbon;  $\text{Ca}^{2+}$ , calcium; CFXS,  $\text{Ca}^{2+}$  concentration in the fruit peduncle xylem sap; CWR, carbon weight loss due to respiration; DAP, day after pollination; DW, dry weight; ECA, estimated  $\text{Ca}^{2+}$  accumulation; HRM, heat ratio method; PSC, phloem sap solutes concentration; PSU, phloem sap uptake; QCA, quantified  $\text{Ca}^{2+}$  accumulation; SWP, stem water potential; Ts, transpiration; VPD, vapour pressure deficit; WUG, water uptake for growth; XSU, xylem sap uptake.

dark brown as cells die (Suzuki *et al.*, 2003; De Freitas *et al.*, 2010).

Although  $\text{Ca}^{2+}$  is believed to move in the plant exclusively through the xylem vascular tissue (Ho *et al.*, 1993; Taylor and Locascio, 2004), the mechanisms regulating  $\text{Ca}^{2+}$  partitioning and allocation in tomato plants and fruit remain poorly understood. Consistent with xylem sap flow, the direction and rate of xylemic  $\text{Ca}^{2+}$  flow in the plant should be determined by water potential gradients in response to different tissue transpiration and growth rates (Taylor and Locascio, 2004; Ho and White, 2005). In that case, higher transpiration and growth rates can reduce water potential and increase tissue strength as sinks for xylemic  $\text{Ca}^{2+}$ . Therefore, the partitioning of  $\text{Ca}^{2+}$  flowing from the roots toward leaves and fruit will depend on the xylem sap  $\text{Ca}^{2+}$  concentration, as well as leaf and fruit transpiration and growth rates. Accordingly, leaves have much higher transpiration rates than fruit, which results in much higher  $\text{Ca}^{2+}$  content in the leaves than in the fruit (Ho, 1989; Ho and White, 2005; De Freitas *et al.*, 2011b).

Previous studies have shown that specifically reducing leaf transpiration by decreasing atmospheric vapour pressure deficit (VPD) or treating tomato plants with abscisic acid (ABA) can potentially decrease xylemic  $\text{Ca}^{2+}$  movement into the leaves, and increase its movement into the fruit (Guichard *et al.*, 2005; De Freitas *et al.*, 2011b). However, direct measurements of xylemic  $\text{Ca}^{2+}$  concentration and xylem sap flow rates into leaves and fruit in response to reduced leaf transpiration rates have not been reported.

Spraying whole plants with ABA increases fruit total tissue and apoplastic  $\text{Ca}^{2+}$  concentrations, and reduced fruit cell membrane leakage and the incidence of blossom-end rot (BER) (De Freitas *et al.*, 2011b). These studies suggest that ABA may affect not only total fruit tissue  $\text{Ca}^{2+}$  concentration but also the regulation of cellular  $\text{Ca}^{2+}$  distribution, which could affect fruit susceptibility to  $\text{Ca}^{2+}$  deficiency disorders such as BER (Park *et al.*, 2005; De Freitas *et al.*, 2011a, 2012). Since these studies were based on whole-plant ABA sprays, the results cannot be specifically attributed to whole-plant or fruit responses to ABA (De Freitas *et al.*, 2011b). Fruit-specific ABA studies are still needed to understand if the prevention of BER development is a whole-plant, a fruit-specific, or a combination response to ABA.

The objectives of this study were to determine  $\text{Ca}^{2+}$  partitioning and allocation in tomato plants and fruit in response to whole-plant and fruit-specific ABA treatments, as well as to analyse the effect of changes in  $\text{Ca}^{2+}$  partitioning and allocation on fruit susceptibility to BER under water stress conditions.

## Materials and methods

Tomato plants [*Solanum lycopersicum* cultivar Ace 55 (Vf)] were grown in 9.5 litre pots in a greenhouse environment with day/night temperature of 27 °C/20 °C and relative humidity of 64%/78%. The plants were grown in an organic substrate of equal portions of peat, sand, and redwood compost, with 2.6 g kg<sup>-1</sup> dolomite lime. Plants were irrigated once a day at about 08:00h with a nutrient solution containing N (102mg l<sup>-1</sup>), P (26mg l<sup>-1</sup>), K (124mg l<sup>-1</sup>), Ca (90mg

l<sup>-1</sup>), Mg (24mg l<sup>-1</sup>), S (16mg l<sup>-1</sup>), Fe (1.6mg l<sup>-1</sup>), Mn (0.27mg l<sup>-1</sup>), Cu (0.16mg l<sup>-1</sup>), Zn (0.12mg l<sup>-1</sup>), B (0.26mg l<sup>-1</sup>), and Mo (0.016mg l<sup>-1</sup>). At full bloom, fully open flowers in the first cluster were tagged and manually pollinated. After pollination, plants were irrigated once a day at 08:00h with 450 ml of the same nutrient solution, but without  $\text{Ca}^{2+}$ , for the remaining period of the study to induce BER development in the fruit. The volume of nutrient solution added daily to the plants after pollination was determined from the volume of total plant water loss per day determined as described below (~450 ml plant<sup>-1</sup> d<sup>-1</sup>). This approach was used to induce plant hydration followed by plant dehydration in a 24h cycle. Wilting was typically observed in the control plants prior to irrigation in the morning, but plants recovered turgor upon irrigation. The water stress condition was used to enhance fruit susceptibility to BER.

Beginning 1 day after pollination (DAP), each plant was sprayed weekly with 200 ml of deionized water (control) or 500 mg l<sup>-1</sup> ABA (Valent Biosciences, Libertyville, IL, USA), or 4–6 fruit on separate plants from those sprayed were dipped weekly in 300 ml of deionized water (control) or 500 mg l<sup>-1</sup> ABA (Valent Biosciences). All solutions contained 0.5 ml l<sup>-1</sup> of Silwet L-77 (Helena Chemical Co., Collierville, TN, USA) as a surfactant. The dipping treatments were applied by completely immersing the fruit in each solution for a period of 10 s. All treatments were applied once a week until 30 DAP. Each treatment was composed of four single plant replications at each evaluation time. After evaluation, plants were eliminated from the experiment. On each plant, 6–8 flowers on the first cluster were tagged, resulting in 6–8 tagged fruit per replication. No thinning treatment was applied to non-tagged flowers or fruit. Plants and fruit were analysed at 15 and 30 DAP as described below. Fruit sampling was accomplished at the end of the irrigation cycle, before 08:00h.

### Relative humidity and air temperature

The relative humidity and air temperature inside the greenhouse were monitored daily every 15 min. The results presented are the hourly averages from 15 to 30 DAP. VPD was calculated based on the hourly measured relative humidity and air temperature as described by Otieno *et al.* (2012).

### Percentage blossom-end rot incidence

The percentage BER was determined by dividing the number of tagged fruit with BER symptoms by the total number of tagged fruit per plant and multiplying this value by 100.

### Electrolyte leakage

Analysis of electrolyte leakage was carried out according to the method described by De Freitas *et al.* (2011a). Briefly, three fruit discs of 1 cm diameter and 0.7 cm thickness were cut from the blossom end of healthy fruit and sectioned with a double-bladed knife 1 mm under the skin. Each sample of three discs from three fruit represented one replication, which was placed into a 50 ml conical tube on a rotary shaker in a 0.2 M mannitol solution. The conductivity of the mannitol solution was recorded periodically over 6 h. Then, samples were frozen and thawed twice to determine the total conductivity (Saltveit, 2002). The results were expressed as the percentage increase in electrolyte leakage (conductivity) per gram of tissue per hour relative to the total tissue conductivity.

### Stem water potential (SWP)

The SWP was determined from 13:00h to 15:00h by bagging one leaf directly connected to the main stem in a reflective envelope for ~20 min. Each bagged leaf was then cut from the plant and placed inside a pressure chamber (PMS Instrument Company, Albany, OR, USA) for SWP measurement (McCutchan and Shackel, 1992).

#### Leaf stomatal conductance

This was analysed in two opposite, fully expanded leaves located on the base, middle, and top regions of each tomato plant. The base, middle, and top regions were defined by visually dividing the height of the plant into three equal sections. The stomatal conductance measurements were accomplished from 13:00h to 15:00h using a steady-state porometer (LI-1600; LI-COR Biotechnology, Lincoln, NE, USA).

#### Total plant water loss

The total water loss was determined by irrigating the plants early in the morning. After draining, the pots were bagged and weighed. Twenty-four hours later, the pots were weighed again and the volume of water lost was calculated as the difference between the first and the last weight per plant.

#### Xylem sap flow measurements

These were accomplished using the heat ratio method (HRM) with an external sap flow sensor (Burgess *et al.*, 2001; Green *et al.*, 2003; Clearwater *et al.*, 2009). The HRM was developed to measure low net sap flow rates that can take place in either direction in the vascular tissue (Burgess *et al.*, 2001; Green *et al.*, 2003; Clearwater *et al.*, 2009), but for the current study only the xylem sap flow rate was determined by heat girdling the middle leaf pedicel or fruit peduncle. The heat girdling was accomplished by passing an electrical signal (0.8 A, 10 V) for 20 s across a constantan wire with 0.8 mm diameter looped twice around the pedicel or peduncle 1 cm upstream of each heat sensor before starting the sap flow measurements (Else *et al.*, 1996). Heat girdling destroys the phloem cells, obstructing phloem sap movement, while the xylem sap flow remains intact and functional due to its non-living cells. This technique has been used to isolate and quantify phloem and xylem sap flow rates (Else *et al.*, 1996; Guichard *et al.*, 2005; Peuke *et al.*, 2006; Clearwater *et al.*, 2009; Hossain and Nonami, 2010). After heat girdling, sap flow measurements were made over a 24 h period. After xylem sap flow measurements, zero sap flow readings were determined by cutting the middle leaf pedicel or fruit peduncle 1 cm downstream of each sensor. The zero xylem sap flow readings were used to determine the baseline accurately for each sap flow sensor after sap flow measurements. After determining the zero sap flow rate, the middle leaf pedicel or fruit peduncle was cut at the heat sensor region to measure the diameter of the xylem vascular tissue, which was used to calculate the volume of xylem sap moving into the leaf and fruit over time. One fully expanded top leaf and one tagged fruit on each plant replication were used for the sap flow analysis. All sensor signals were logged (CR10X, Campbell Scientific Inc., Logan, UT, USA) at 20 min intervals and averaged every hour. The results presented are the averages of four replications. Xylem sap uptake (XSU) into the fruit was determined based on the daily average xylem sap flow measurements from 15 and 30 DAP.

#### Extraction of soil solution, xylem sap, and apoplastic solution

Extractions were accomplished at the end of the irrigation cycle, before 08:00h. Soil solution was extracted by adding 450 ml of the nutrient solution, without  $\text{Ca}^{2+}$ , to each plant pot, and collecting the drained leachate. After collecting the soil leachate, plants were decapitated 15 cm above the soil level and the pots containing the roots plus stem stump were used to extract the stem xylem sap. Two fruit and two middle leaves were harvested at the end of the irrigation cycle from each replicate plant with the entire peduncle and pedicel attached and kept in a sealed plastic bag for xylem sap extraction. Xylem sap extraction was accomplished by placing the plant pot, fruit, or leaf blade inside a pressure chamber (PMS Instrument Company), while the cut end of the stem, peduncle, or pedicel was exposed to the outside of the chamber through a seal.

After sealing, the pressure inside the chamber was increased up to 0.8 MPa with  $\text{N}_2$ . The initial xylem sap moving out of the stem, peduncle, or pedicel cut end was blotted dry to reduce  $\text{Ca}^{2+}$  contamination from the cut. The following 100  $\mu\text{l}$  was collected over a period of 5 min and used to determine the  $\text{Ca}^{2+}$  concentration in the xylem sap (Wartinger *et al.*, 1990; Schurr and Schulze, 1995; Montanaro *et al.*, 2006). Fruit and leaf were pressurized inside a commercial pressure chamber (PMS Instrument Company). The plant pots were pressurized in a custom-built chamber large enough to hold a 9.5 litre pot, with a two-part (split) lid in order to allow assembly around the stem of an intact plant (PMS Instrument Company). The custom-built chamber was also used to pressurize the roots of whole plants to induce guttation on leaf blades, which were collected for  $\text{Ca}^{2+}$  quantification. The guttation samples represent the xylem sap extracted from leaf blades without any contamination from a cut surface. Apoplastic water-soluble  $\text{Ca}^{2+}$  was extracted from the blossom-end pericarp tissue of tomato fruit as previously described by De Freitas *et al.* (2011a).

#### Calcium concentration

The  $\text{Ca}^{2+}$  concentration in the soil solution, xylem sap, and apoplastic solution was determined with an Ultra-M micro  $\text{Ca}^{2+}$ -selective electrode (Lazar Research Laboratories, Inc., Los Angeles, CA, USA). A standard  $\text{Ca}^{2+}$  calibration curve ( $R^2=0.98$ ) was used to determine the  $\text{Ca}^{2+}$  concentration in the samples.

The  $\text{Ca}^{2+}$  concentration in leaf and fruit tissues was determined in freeze-dried leaf blades, as well as pericarp tissues manually cut from the peduncle and blossom regions of the fruit. Freeze-dried samples were subjected to microwave acid digestion and analysed by inductively coupled plasma atomic emission spectrometry (Meyer and Keliher, 1992). Calcium accumulation was quantified by subtracting the total middle leaf and fruit  $\text{Ca}^{2+}$  contents observed at 15 DAP from the total middle leaf and fruit  $\text{Ca}^{2+}$  contents observed at 30 DAP. Calcium accumulation was also estimated by multiplying the quantified xylem sap  $\text{Ca}^{2+}$  concentration observed in the middle leaf pedicel and fruit peduncle by its respective daily average xylem sap flow rate observed at 15 and 30 DAP.

#### Xylem function

This was measured in developing fruit as previously described by Ho *et al.* (1993) and De Freitas *et al.* (2011b). Fruits were harvested and held in sealed plastic bags for 20 min with 100 ml of free water to reduce transpiration until the peduncle of each fruit was immersed in a solution of 1% Safranin-O at 20 °C under  $\leq 20\%$  relative humidity. After 24 h, fruit were cut into three equal sections at a 90 ° angle to the peduncle axis. The number of stained vascular bundles (xylem vessels) was counted in the placenta and pericarp tissues at the cut surfaces at the blossom and peduncle end regions of each fruit.

#### Fruit growth rate

The rate of fruit growth was measured over a 24 h period using dendrometers (Model DEX20, Dynamax Inc., Houston, TX, USA). One tagged fruit on each plant replication was used for fruit growth rate analysis. All sensor signals were logged (CR1000, Campbell Scientific Inc.) at 10 min intervals and averaged every hour.

#### Fruit weight

The fruit weight was determined by dividing the total weight of all tagged fruit on the plant by the total number of tagged fruit on each plant replication. The final result represents the average of all replications in each treatment. The average fruit water uptake for growth (WUG) was determined by subtracting the fruit water content quantified at 15 DAP from the fruit water content quantified at 30 DAP and dividing that value by 15 d. The results are presented as volume of sap uptake per fruit per day.



*Phloem sap uptake (PSU)*

Analysis of the PSU was based on the assumption that the total fruit water uptake from 15 to 30 DAP was supplied only by xylem and phloem. Therefore, PSU was determined as the sum of WUG plus transpiration (Ts) minus XSU. In this case,

$$PSU = (WUG + Ts) - XSU$$

Based on previous studies,  $Ts = 375 \mu\text{l}$  of water  $\text{fruit}^{-1} \text{d}^{-1}$ , for the same tomato fruit developmental stage (Liu *et al.*, 2007). The values obtained for the calculations represent the average from 15 to 30 DAP. The results were expressed as  $\mu\text{l}$  of sap uptake  $\text{fruit}^{-1} \text{d}^{-1}$ . Phloem sap solutes concentration (PSC) was calculated as the fruit dry weight (DW) accumulated from 15 to 30 DAP plus the carbon (C) weight loss due to fruit respiration (CWR) divided by the PSU from 15 to 30 DAP (Liu *et al.*, 2007; Génard and Lescouret, 2012). In this case,

$$PSC = (DW + CWR) / PSU$$

According to previous studies,  $CWR = 0.0895 \text{ g C fruit}^{-1} \text{d}^{-1}$  for the same tomato fruit developmental stage (Liu *et al.*, 2007).

*Statistical analysis*

Analysis of variance (ANOVA) was performed for each variable using the Statistical Analysis System (SAS) software package. The mean values were compared by Tukey's test ( $P=0.05$ ) or presented as means  $\pm$  standard deviation (SD).

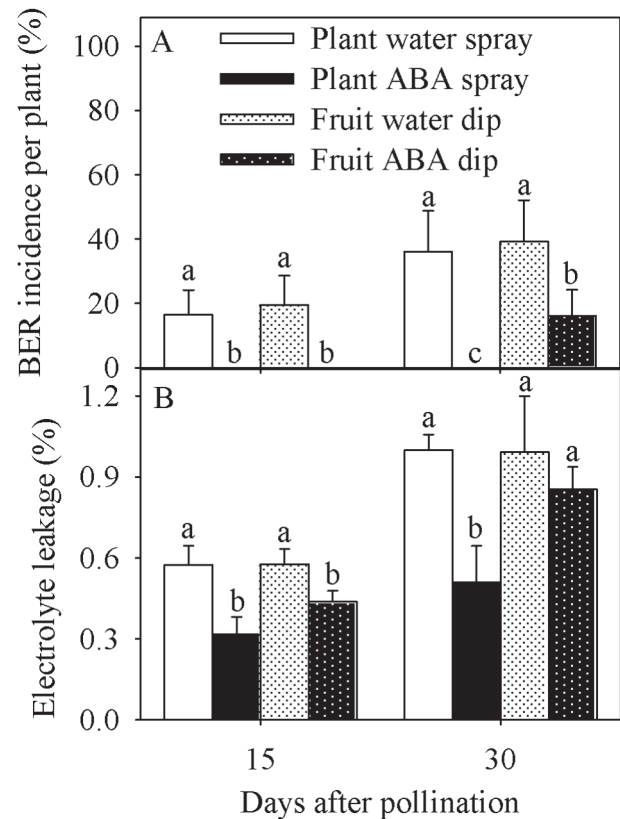
**Results**

BER was completely suppressed by spraying the whole plants weekly with ABA during fruit growth and development, compared with water-sprayed fruit that reached a 36% incidence of BER at 30 DAP (Fig. 1A). Dipping the fruit in solutions containing ABA prevented BER development at 15 DAP, but ABA-dipped fruit reached a 16% incidence of BER at 30 DAP. Control fruit dipped in water had a 39% incidence of BER at 30 DAP. The electrolyte leakage of fruit pericarp tissue was lower in response to whole-plant and fruit-specific ABA treatments at 15 DAP (Fig. 1B). At 30 DAP, only the whole-plant ABA treatment had lower electrolyte leakage in fruit pericarp tissue.

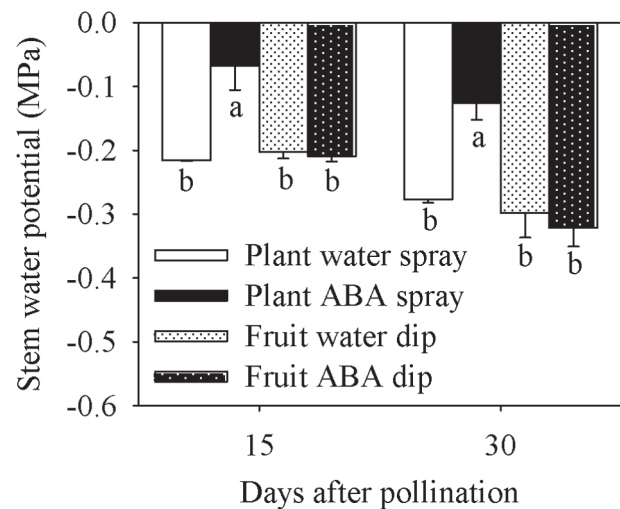
SWP was less negative in response to whole-plant ABA treatment at 15 and 30 DAP compared with all other treatments (Fig. 2). Leaf stomatal conductance progressively increased from the base, middle, to the top regions of the plant, and was lower in the whole-plant ABA treatment at 15 and 30 DAP compared with all other treatments (Fig. 3). Whole-plant water spraying, as well as water and ABA dip treatments, had similar stomatal conductance for the basal, middle, and top leaves. Based on the stomatal conductance analysis, the plant ABA uptake was considered high with whole-plant ABA treatment, and no significant ABA movement from the fruit into the plant was observed based on changes in stomatal conductance in response to fruit-specific ABA dip treatment (Fig. 3).

Plant water loss was reduced by the whole-plant ABA treatment at 15 and 30 DAP (Fig. 4), but was similar in all other treatments, including the ABA fruit dip. Whole-plant

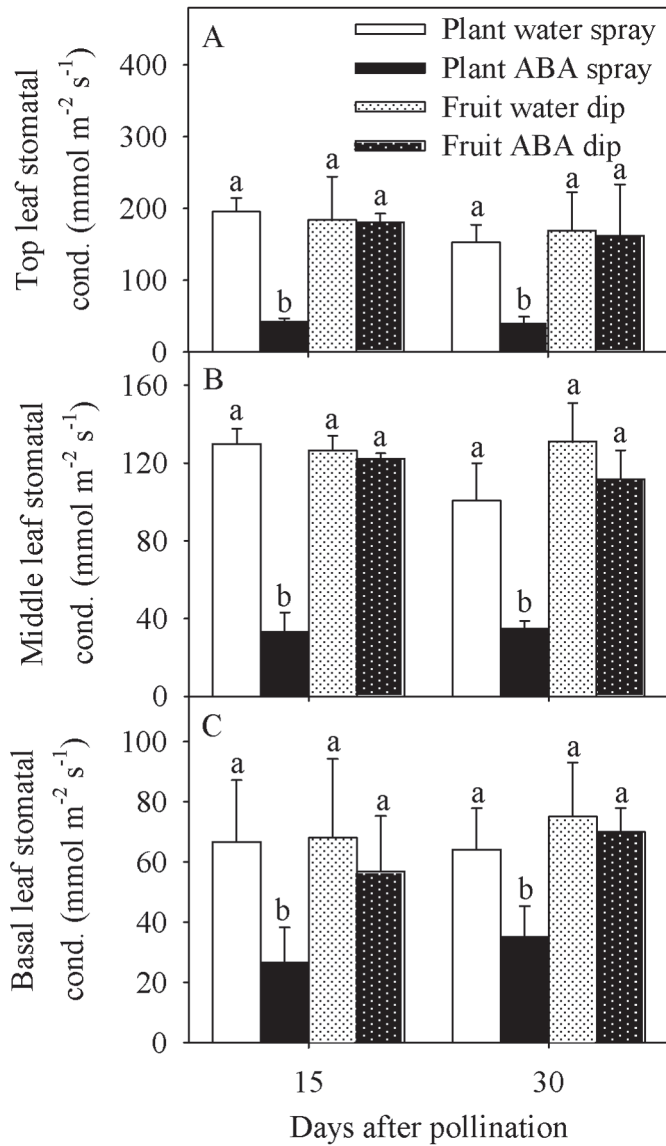
ABA treatment maintained a significantly lower xylem sap flow rate into the top leaves compared with all other treatments, especially during the daylight hours over a 24 h period



**Fig. 1.** Blossom-end rot incidence (A) and electrolyte leakage (B) of tomato fruit of the Ace 55 (Vf) cultivar at 15 and 30 days after pollination (DAP). Vertical bars indicate means  $\pm$ SD,  $n=4$  for all treatments. Means with the same letter at each evaluation time are not significantly different ( $P=0.05$ ) according to Tukey's test.



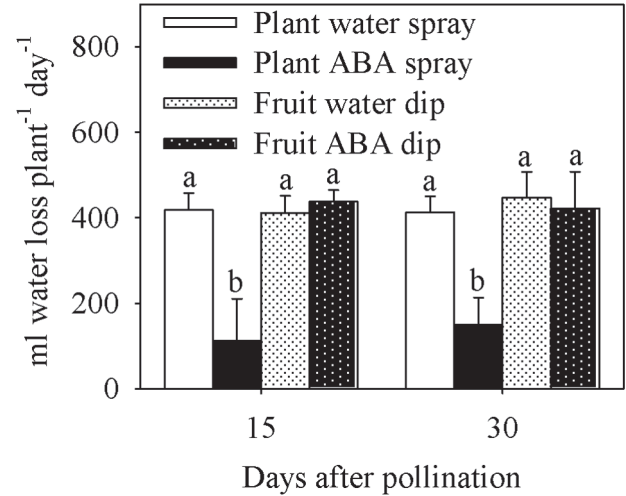
**Fig. 2.** Midday stem water potential (SWP) of tomato plants of the Ace 55 (Vf) cultivar at 15 and 30 DAP. Vertical bars indicate means  $\pm$ SD,  $n=4$  for all treatments. Means with the same letter at each evaluation time are not significantly different ( $P=0.05$ ) according to Tukey's test.



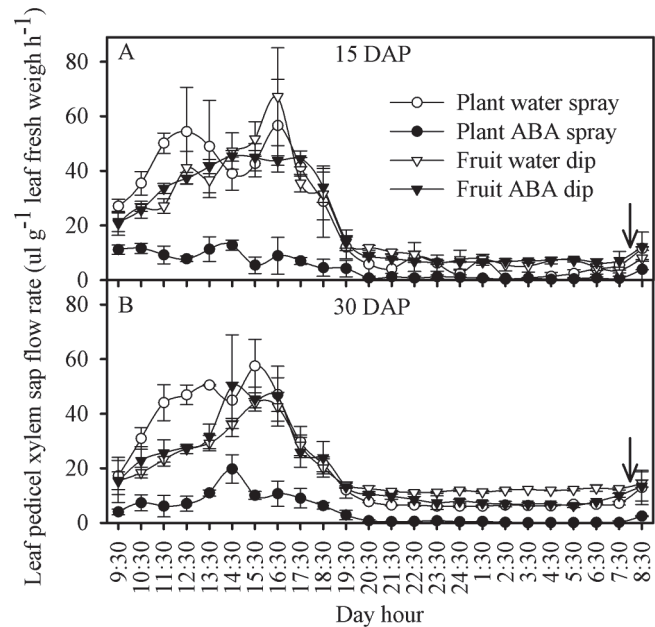
**Fig. 3.** Top (A), middle (B), and basal (C) leaf stomatal conductance of tomato plants of the Ace 55 (Vf) cultivar at 15 and 30 DAP. Vertical bars indicate means  $\pm$ SD,  $n=4$  for all treatments. Means with the same letter at each evaluation time are not significantly different ( $P=0.05$ ) according to Tukey's test.

at 15 and 30 DAP (Fig. 5, Table 1). The water spray treatment had the highest sap flow during most of the daylight period at 15 and 30 DAP (Fig. 5).

The average flow of xylem sap moving into the fruit during a 24h irrigation cycle was substantially higher on plants sprayed with ABA, compared with all other treatments (Fig. 6, Tables 1, 2), with the same diurnal pattern as seen in the leaves (Fig. 5). At 15 DAP, fruit on water-sprayed plants, as well as water- and ABA-dipped fruit had a reverse flow of xylemic sap from the fruit back to the plant, starting in the late afternoon until the next irrigation cycle in the morning (Fig. 6A). The ABA-dipped treatment had a slightly higher sap flow to the fruit than the two water treatments at 15 and at 30 DAP (Table 1). Fruit on plants sprayed with ABA had no reverse xylemic sap flow throughout the irrigation cycle



**Fig. 4.** Daily water loss of tomato plants of the Ace 55 (Vf) cultivar at 15 and 30 DAP. Vertical bars indicate means  $\pm$ SD,  $n=4$  for all treatments. Means with the same letter at each evaluation time are not significantly different ( $P=0.05$ ) according to Tukey's test.



**Fig. 5.** Sap flow rate in the top leaf pedicel of tomato plants of the Ace 55 (Vf) cultivar at 15 (A) and 30 (B) DAP. Vertical bars indicate means  $\pm$ SD,  $n=4$  for all treatments. The arrow represents the time of irrigation.

at 15 DAP, but had the same diurnal pattern, with the lowest flows occurring during the night period (Fig. 6A). The diurnal pattern of fruit xylem flow at 30 DAP was similar to that at 15 DAP, but the magnitude was substantially reduced, again with no discernible reverse flow (Fig. 6B, Tables 1, 2). Spraying tomato plants with ABA resulted in higher total fruit water uptake used for growth and lower fruit water uptake through the phloem from 15 to 30 DAP (Table 2). The estimated phloem sap solute concentration uptake into the fruit from 15 to 30 DAP was higher in ABA-sprayed plants than in non-sprayed plants (Table 2).

The  $\text{Ca}^{2+}$  concentrations in the soil solution and in the main stem xylem sap were similar among all treatments at 15 and 30 DAP. The average  $\text{Ca}^{2+}$  concentration in the soil solution among treatments was  $1.41 \pm 0.09 \text{ mM}$  at 15 DAP and  $1.08 \pm 0.14 \text{ mM}$  at 30 DAP. The average  $\text{Ca}^{2+}$  concentration in the main stem xylem sap was  $0.72 \pm 0.04 \text{ mM}$  at 15 DAP and  $0.63 \pm 0.05 \text{ mM}$  at 30 DAP. There was no statistical difference among treatments in  $\text{Ca}^{2+}$  concentrations in the xylem sap of basal, middle, or top leaves at 15 or 30 DAP. The same results were obtained when  $\text{Ca}^{2+}$  was determined on an independent set of plants under the same treatments using the leaf guttation method (data

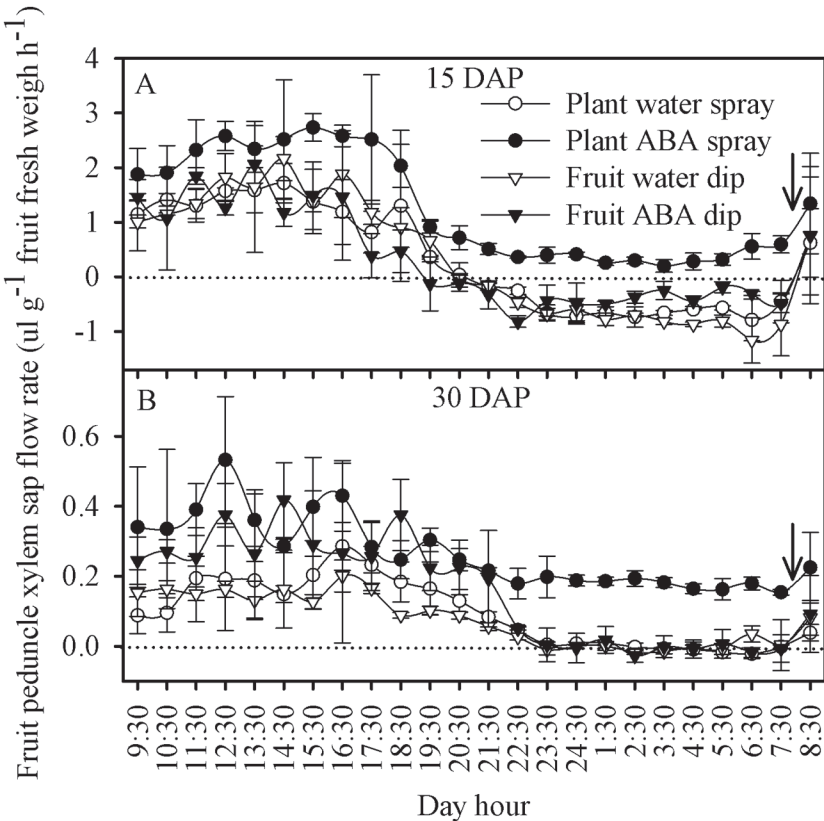
**Table 1.** Average xylemic sap flow rate in leaf pedicel and fruit peduncle of tomato cultivar Ace 55 (Vf) at 15 and 30 DAP

Treatment	Leaf pedicel ( $\mu\text{l g}^{-1}$ leaf FW $\text{h}^{-1}$ )		Fruit peduncle ( $\mu\text{l g}^{-1}$ fruit FW $\text{h}^{-1}$ )	
	15 DAP	30 DAP	15 DAP	30 DAP
Plant water spray	20.7 a*	20.6 a	0.34 b	0.09 c
Plant ABA spray	4.4 b	4.2 b	1.27 a	0.27 a
Fruit water dip	20.5 a	19.0 a	0.33 b	0.08 c
Fruit ABA dip	20.2 a	18.2 a	0.36 b	0.16 b
CV (%)	19.8	18.2	14.6	5.4

\*Means with the same letter between treatments at each evaluation time are not significantly different ( $P=0.05$ ) according to Tukey's test. CV, coefficient of variation.

not shown). The average xylem sap  $\text{Ca}^{2+}$  concentrations were  $0.71 \pm 0.06 \text{ mM}$  and  $0.86 \pm 0.02 \text{ mM}$  in top leaves,  $0.77 \pm 0.03 \text{ mM}$  and  $0.87 \pm 0.02 \text{ mM}$  in middle leaves, and  $0.64 \pm 0.03 \text{ mM}$  and  $0.81 \pm 0.02 \text{ mM}$  in basal leaves at 15 and 30 DAP, respectively. The  $\text{Ca}^{2+}$  concentration in the peduncle xylem sap was higher in fruit from ABA-sprayed plants at 15 and 30 DAP, compared with all other treatments (Table 3), and water-soluble apoplastic  $\text{Ca}^{2+}$  was higher in fruit from ABA-sprayed plants at 15 and 30 DAP, compared with all other treatments (Table 3). Fruit dipped in ABA solution had slightly higher water-soluble apoplastic  $\text{Ca}^{2+}$  than fruit dipped in water and fruit from plants sprayed with water at 15 DAP.

The  $\text{Ca}^{2+}$  concentration in the top and middle leaves was statistically lower in response to whole-plant ABA treatment compared with all other treatments at 15 and 30 DAP (Table 6). The  $\text{Ca}^{2+}$  concentrations in ABA-sprayed plants were  $8.7 \pm 0.21$  and  $8.1 \pm 0.09 \text{ mg g DW}^{-1}$  in top leaves and  $17.5 \pm 0.52$  and  $16.1 \pm 0.63 \text{ mg g DW}^{-1}$  in middle leaves at 15 and 30 DAP, respectively. The  $\text{Ca}^{2+}$  concentrations in all other non-ABA-sprayed plants were  $13.0 \pm 0.36$  and  $13.0 \pm 0.15 \text{ mg g DW}^{-1}$  in top leaves and  $25.1 \pm 0.96$  and  $23.9 \pm 0.81 \text{ mg g DW}^{-1}$  in middle leaves at 15 and 30 DAP, respectively. The  $\text{Ca}^{2+}$  concentration in basal leaves was similar in all treatments at 15 DAP (average= $36.7 \pm 1.5 \text{ mg g DW}^{-1}$ ), and statistically lower in plants sprayed with ABA (average= $27.4 \pm 0.42 \text{ mg g DW}^{-1}$ ) than all other treatments (average= $32.8 \pm 0.53 \text{ mg g DW}^{-1}$ ) at 30 DAP. The  $\text{Ca}^{2+}$  concentration in fruit tissue collected at the



**Fig. 6.** Sap flow rate in fruit peduncle of tomato cultivar Ace 55 (Vf) at 15 (A) and 30 (B) DAP. Vertical bars indicate means  $\pm$ SD,  $n=4$  for all treatments. The arrow represents the time of irrigation. Note the difference in the y-scale.

**Table 2.** Average fruit water uptake for growth, fruit water uptake through the xylem and phloem vessels, and phloem solute concentration from 15 to 30 DAP

Treatment	Growth water uptake ( $\mu\text{l fruit}^{-1} \text{d}^{-1}$ )	Xylem sap uptake ( $\mu\text{l fruit}^{-1} \text{d}^{-1}$ )	Phloem sap uptake ( $\mu\text{l fruit}^{-1} \text{d}^{-1}$ )	Phloem solutes ( $\text{mg ml}^{-1}$ )
Plant water spray	964 b*	222 b	1117 a	132 b
Plant ABA spray	1450 a	1046 a	778 b	301 a
Fruit water dip	800 b	189 b	986 a	146 b
Fruit ABA dip	902 b	259 b	1018 a	155 b
CV (%)	11.5	10.2	10.8	10.9

\*Means with the same letter between treatments at each evaluation time are not significantly different ( $P=0.05$ ) according to Tukey's test. CV, coefficient of variation.

**Table 3.** Calcium concentration in the xylem sap of fruit peduncle and in the apoplast of blossom-end pericarp tissue of the tomato fruit Ace 55 (Vf) cultivar at 15 and 30 DAP

Treatment	Fruit peduncle xylem $\text{Ca}^{2+}$ (mM)		Fruit blossom-end apoplastic $\text{Ca}^{2+}$ (mM)	
	15 DAP	30 DAP	15 DAP	30 DAP
Plant water spray	0.73 b*	1.00 b	0.04 c	0.13 b
Plant ABA spray	0.97 a	1.24 a	0.27 a	0.46 a
Fruit water dip	0.71 b	0.87 b	0.04 c	0.11 b
Fruit ABA dip	0.71 b	0.86 b	0.09 b	0.18 b
CV (%)	12.3	11.8	24.2	27.5

\*Means with the same letter between treatments at each evaluation time are not significantly different ( $P=0.05$ ) according to Tukey's test. CV, coefficient of variation.

peduncle and blossom ends of the fruit was higher in ABA-sprayed plants at 15 and 30 DAP (Table 4). Fruit dipped in ABA had a higher  $\text{Ca}^{2+}$  concentration at the blossom-end tissue at 15 DAP, compared with water-dipped fruit and fruit of water-sprayed plants (Table 4).  $\text{Ca}^{2+}$  accumulation was lower in the leaf and higher in the fruit of ABA-sprayed plants than in the other treated plants and fruit from 15 to 30 DAP (Table 5).  $\text{Ca}^{2+}$  accumulation in leaf and fruit quantified by tissue analysis was similar to the estimated  $\text{Ca}^{2+}$  accumulation based on the  $\text{Ca}^{2+}$  concentration in the xylem sap and xylem sap flow rates into leaf and fruit tissues (Table 5). The average relative humidity and air temperature from 15 to 30 DAP inside the greenhouse, where the tomato plants were grown, oscillated from 58.2% and 27.8 °C during the day up to 77.8% and 18.2 °C during the night, respectively (Fig. 7). The VPD increased from 0.5 kPa at 05:30h to 1.6 kPa at 14:30h, decreasing thereafter (Fig. 7).

The number of Safranin-O-stained vascular bundles in the placenta and pericarp tissues at the peduncle and blossom-end regions of the fruit was higher in response to whole-plant and fruit-specific ABA treatments at 15 DAP (Fig. 8). The number of stained vascular bundles decreased in all treatments from 15 to 30 DAP, and all treatments showed a similar number of stained vascular bundles in the placenta and pericarp tissues at the peduncle and blossom-end regions of the fruit at 30 DAP (Fig. 8).

**Table 4.** Calcium concentration observed in the peduncle end and blossom-end tissues of tomato fruit of the Ace 55 (Vf) cultivar at 15 and 30 DAP

Treatment	Fruit peduncle end ( $\text{mg Ca}^{2+} \text{g DW}^{-1}$ )		Fruit blossom end ( $\text{mg Ca}^{2+} \text{g DW}^{-1}$ )	
	15 DAP	30 DAP	15 DAP	30 DAP
Plant water spray	0.74 b*	0.70 b	0.34 c	0.25 b
Plant ABA spray	1.41 a	1.14 a	0.80 a	0.59 a
Fruit water dip	0.66 b	0.59 b	0.30 c	0.26 b
Fruit ABA dip	0.69 b	0.57 b	0.48 b	0.25 b
CV (%)	8.7	18.7	8.6	11.4

\*Means with the same letter between treatments at each evaluation time are not significantly different ( $P=0.05$ ) according to Tukey's test. CV, coefficient of variation.

The fruit growth rate was higher in ABA-sprayed plants compared with all other treatments at 15 and 30 DAP (Fig. 9). All treatments showed a positive fruit growth rate during a 24h period at 15 and 30 DAP (Fig. 9). For all treatments, the fruit growth rate was higher at 15 DAP than at 30 DAP (Fig. 9). The average fruit weight was also higher in ABA-sprayed plants at 15 and 30 DAP (Fig. 10). Fruit  $\text{Ca}^{2+}$  uptake, both directly quantified and estimated based on the product of fruit xylem sap uptake and fruit peduncle xylem sap  $\text{Ca}^{2+}$  concentration, was 6-fold higher in ABA-sprayed plants compared with water-sprayed controls (Table 6). A much smaller increase in  $\text{Ca}^{2+}$  uptake was found in ABA-dipped fruit, but, again, this was consistent for both directly quantified and estimated values (Table 6). The sprayed and dipped ABA/water ratios for fruit growth rate were 1.41 and 1.15, respectively (Table 6).

## Discussion

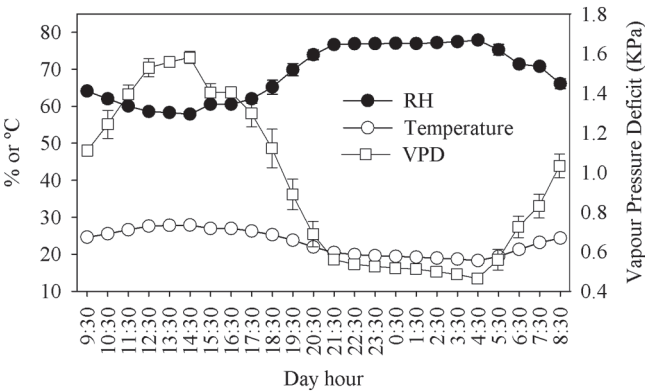
Previous studies showed that weekly spraying of tomato plants with ABA prevented BER development in the fruit, while water-sprayed plants reached a 30–45% incidence of BER at 40–45 DAP (De Freitas *et al.*, 2011b). At that time, possible mechanisms through which ABA increased fruit  $\text{Ca}^{2+}$  concentration and reduced fruit susceptibility to BER were suggested based on estimations of fruit xylem sap uptake and  $\text{Ca}^{2+}$  concentration in the xylem sap (De Freitas *et al.*, 2011b).



**Table 5.** Quantified (QCA) and estimated (ECA)  $\text{Ca}^{2+}$  accumulation in leaf and fruit of the tomato Ace 55 (Vf) cultivar from 15 to 30 DAP

Treatment	$\mu\text{g Ca}^{2+} \text{ leaf}^{-1} \text{ d}^{-1}$			$\mu\text{g Ca}^{2+} \text{ fruit}^{-1} \text{ d}^{-1}$		
	QCA	ECA <sup>a</sup>	Ratio <sup>b</sup>	QCA	ECA <sup>a</sup>	Ratio <sup>b</sup>
Plant water spray	203.4 a*	201.6 a	0.99	8.3 b	7.1 b	0.85
Plant ABA spray	40.3 b	39.9 b	0.99	49.8 a	43.1 a	0.86
Fruit water dip	197.7 a	195.8 a	0.99	7.1 b	5.6 b	0.79
Fruit ABA dip	196.6 a	186.7 a	0.94	9.1 b	7.9 b	0.86
CV (%)	8.8	13.6		11.8	11.0	

\*Means with the same letter between treatments are not significantly different ( $p=0.05$ ) according to Tukey's test.  
<sup>a</sup>Calcium accumulation was estimated by multiplying the xylem sap  $\text{Ca}^{2+}$  concentration observed in middle leaf pedicel and fruit peduncle by its respective xylem sap flow rate.  
<sup>b</sup> Ratio=estimated/quantified.  
CV, coefficient of variation.



**Fig. 7.** Average air relative humidity (RH), temperature, and vapour pressure deficit (VPD) in the greenhouse environment during growth and development of cultivar Ace 55 (Vf) tomatoes from 15 to 30 DAP. Vertical bars indicate means  $\pm$ SD.

In the present study, direct measurements of xylem sap uptake and xylem sap  $\text{Ca}^{2+}$  concentration in the main stem, leaf pedicel, and fruit peduncle were used to demonstrate that xylem sap flow and  $\text{Ca}^{2+}$  movement into fruit were substantially increased with spraying the whole plant with ABA.

*Whole-plant  $\text{Ca}^{2+}$  uptake and partitioning in response to ABA*

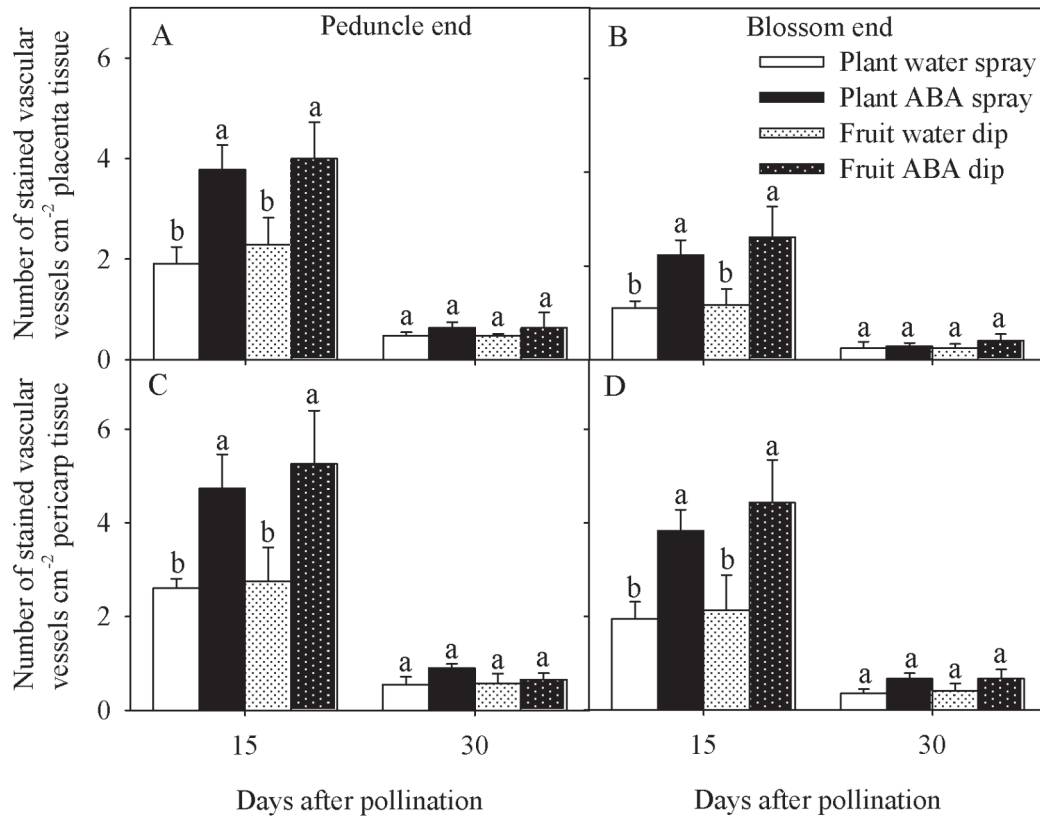
Water uptake in leaves comes exclusively from xylem vessels, while water uptake into the fruit comes from both phloem and xylem vascular tissues (Ho and White, 2005). Treating the whole plant with ABA reduced stomatal conductance, which resulted in lower plant water loss, lower soil water uptake and xylemic water movement into the leaves, as well as higher SWP and increased xylemic water movement into the fruit. Considering that  $\text{Ca}^{2+}$  concentrations in the soil solution and stem xylem sap were similar among all treatments, the observed lower  $\text{Ca}^{2+}$  accumulation in ABA-sprayed plants was due to lower soil solution uptake triggered by lower leaf transpiration rates (Table 5). Our results also estimate a higher solute concentration in the phloem sap moving into the fruit of ABA-sprayed plants (Table 2). Although ABA reduced stomatal conductance and this would be expected to decrease leaf photosynthesis

(Egea *et al.*, 2011), the improved plant water status associated with ABA application may have caused compensatory physiological effects in other areas, such as reduced carbon partitioning to roots and/or improved carbon transport rates, resulting in higher solute concentration in the phloem sap, compared with the other treatments. The non-ABA-sprayed plants had an average fruit phloem sap uptake of  $1.04 \text{ ml fruit}^{-1} \text{ d}^{-1}$  and an average phloem sap solute concentration of  $144.3 \text{ mg ml}^{-1}$  (Table 2). The ABA-sprayed plants had an average fruit phloem sap uptake of  $0.778 \text{ ml fruit}^{-1} \text{ d}^{-1}$  and an average phloem sap solute concentration of  $301 \text{ mg ml}^{-1}$  (Table 2). Therefore, the solute accumulation per day was  $1.04 \times 144.3 = 150.07 \text{ mg fruit}^{-1} \text{ d}^{-1}$  in non-ABA-sprayed plants and  $0.778 \times 301 = 234.18 \text{ mg fruit}^{-1} \text{ d}^{-1}$  in ABA-sprayed plants. Therefore, the results also showed that ABA-sprayed plants had higher phloem solute accumulation per fruit than non-ABA-sprayed plants from 15 to 30 DAP. According to the present data and other studies, ABA could also be acting at the whole-plant level as a signal triggering carbohydrate accumulation and osmotic adjustment in sink organs (Alves and Setter, 2004). In addition, spraying peach fruit with ABA has been shown to increase the activity of sorbitol oxidase, a predominant enzyme in the metabolism of the translocated sugar alcohol sorbitol, which was followed by an enhanced sugar accumulation in the fruit (Kobashi *et al.*, 1999). The higher phloem sap solute concentration in ABA-sprayed plants can decrease fruit apoplastic solute potential, which is then equilibrated by a parallel decline in fruit total water potential (Matthews *et al.*, 1987). Under these conditions, higher fruit solute accumulation can increase the water potential gradient between the fruit and stem, favouring fruit xylem sap uptake (Johnson *et al.*, 1992; Taylor and Locascio, 2004; Ho and White, 2005). Accordingly, the present results show that whole-plant ABA spray treatment not only increased phloem solute accumulation per fruit, but also decreased leaf transpiration, maintaining a higher stem water potential and higher total fruit water uptake, compared with non-ABA-sprayed plants.

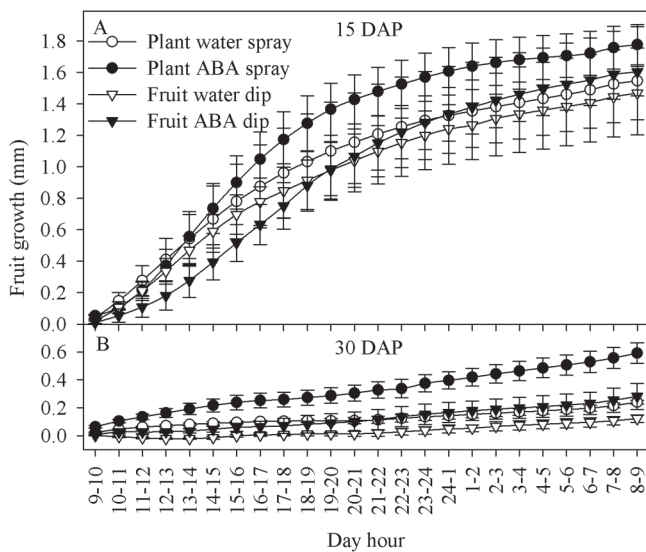
*Leaf xylem sap and  $\text{Ca}^{2+}$  uptake in response to ABA*

Following irrigation in the morning, tomato plants treated with ABA had a smaller increase in xylem sap flow rate into

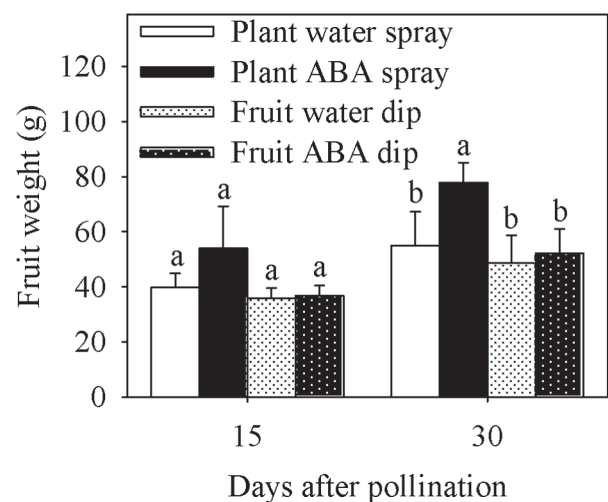




**Fig. 8.** Number of stained vascular bundles in the placenta (A and B) and pericarp (C and D) tissues at the peduncle (A and C) and blossom-end regions (B and D) of Ace 55 (Vf) tomato fruit. Vascular bundles were stained with 1% Safranin-O. Vertical bars indicate means  $\pm$ SD,  $n=4$  for all treatments. Means with the same letter at each evaluation time are not significantly different ( $P=0.05$ ) according to Tukey's test.



**Fig. 9.** Growth rate of tomato fruit of the Ace 55 (Vf) cultivar at 15 and 30 DAP. Vertical bars indicate means  $\pm$ SD,  $n=4$  for all treatments.



**Fig. 10.** Average weight of tomato fruit of the Ace 55 (Vf) cultivar at 15 and 30 DAP. Vertical bars indicate means  $\pm$ SD,  $n=4$  for all treatments. Means with the same letters at each evaluation time are not significantly different ( $P=0.05$ ) according to Tukey's test.

the leaves compared with non-treated plants, presumably due to suppression of stomatal opening (Neill *et al.*, 2008). In all treatments, the increase in xylem sap flow after the time of irrigation in the morning probably reflected the combined effects of leaf rehydration as well as increasing light intensity

stimulating stomatal opening, and increasing VPD from increased air temperatures and decreased relative humidity in the greenhouse environment. The reduction in leaf xylem sap flow after 15:30 h to 16:30 h was presumably a result of the reverse changes in the environmental conditions observed

**Table 6.** Ratios between sprayed or dipped ABA and sprayed or dipped water treatments for quantified  $\text{Ca}^{2+}$  accumulation (QCA), xylem sap uptake (XSU),  $\text{Ca}^{2+}$  concentration in the fruit peduncle xylem sap (CFXS), estimated  $\text{Ca}^{2+}$  accumulation (ECA), and growth rate of tomato fruit of the Ace 55 (Vf) cultivar from 15 to 30 DAP

Analysis	Plant spray			Fruit dip		
	Water	ABA	Ratio <sup>a</sup>	Water	ABA	Ratio
1. QCA ( $\mu\text{g fruit}^{-1} \text{ d}^{-1}$ )	8.30 b*	49.80 a	6.00	7.10 a	9.10 a	1.28
2. XSU ( $\mu\text{l fruit}^{-1} \text{ d}^{-1}$ )	221.5 b	1046.4 a	4.72	189.0 a	259.2 a	1.37
3. CFXS (mM) <sup>b</sup>	0.86 b	1.10 a	1.28	0.79 a	0.78 a	1.00
4. ECA <sup>c</sup> ( $\mu\text{g fruit}^{-1} \text{ d}^{-1}$ )	–	–	6.04	–	–	1.37
5. Fruit growth ( $\text{mm d}^{-1}$ )	1.27 b	1.80 a	1.41	1.07 a	1.23 a	1.15

\*Means with the same letter within spray or dip treatments were not significantly different ( $P=0.05$ ) according to Tukey's test

<sup>a</sup> Ratio=ABA/water.

<sup>b</sup> CFXS=average  $\text{Ca}^{2+}$  concentration in the fruit peduncle xylem sap between 15 and 30 DAP.

<sup>c</sup> Estimated  $\text{Ca}^{2+}$  accumulation (ECA)=Line 2×Line 3.

early in the day after irrigation. These results and other studies also show a direct relationship between high leaf transpiration and higher leaf  $\text{Ca}^{2+}$  uptake (De Freitas *et al.*, 2011b), suggesting that leaf  $\text{Ca}^{2+}$  accumulation is dependent only on leaf xylem sap uptake triggered by leaf transpiration rates. In addition, the data also show that  $\text{Ca}^{2+}$  concentration in the leaf xylem sap extracted by pressurizing the leaves or by inducing leaf guttation were similar, suggesting that there is no significant  $\text{Ca}^{2+}$  contamination of leaf xylem sap when the leaves are cut and pressurized in the pressure chamber for xylem sap extraction.

#### Fruit xylem sap and $\text{Ca}^{2+}$ uptake in response to ABA

The ABA-treated fruit also showed a higher number of stained xylem vessels (potentially functional vessels). In that case, ABA treatment may have also reduced the hydraulic resistance within the fruit, favouring xylemic water movement in the fruit towards the blossom-end tissue, provided a hydrostatic gradient responsible for xylem sap flow was present in the fruit (Bondada *et al.*, 2005). Since  $\text{Ca}^{2+}$  is believed to be mobile in the plant exclusively through the xylem vessels (Ho and White, 2005), the observed increase in xylem sap flow towards the fruit in the pedicle, and a reduced hydraulic resistance within the fruit, may explain the observed higher fruit  $\text{Ca}^{2+}$  accumulation in ABA-treated plants. Neither effect was observed in ABA-dipped fruit, suggesting that changes in  $\text{Ca}^{2+}$  partitioning in the plant are responsive only to whole-plant ABA treatment.

The pattern of fruit xylem sap uptake followed an increase after irrigation in all treatments until 15:30h to 16:30h, decreasing thereafter at both 15 and 30 DAP. Similar to the leaves, this pattern could be explained by the combined effects of an increase in plant water content right after irrigation and an increase in VPD early during the day that increased the evaporative demand due to increasing air temperatures and decreasing relative humidity during the day time. Late in the day, decreasing SWP due to plant water loss and decrease in the VPD and consequently the evaporative demand due to decreasing air temperatures and increasing relative humidity could limit xylem sap flow into the fruit, resulting in

the observed decrease in fruit xylem sap uptake. At night, the VPD was low but not zero, and continued plant water loss under these conditions may have been associated with the observed reverse flow of fruit xylem sap at 15 DAP for non-ABA-treated plants. Although a reverse xylem sap flow was observed later in the irrigation cycle, the fruit growth rate was always positive, indicating that phloem sap uptake maintained the positive growth rates even under reverse xylem sap flow conditions. The reverse flow of fruit xylem sap was not observed at 30 DAP, possibly because of higher fruit solute content compared with 15 DAP. The higher solute content decreased fruit water potential (Bolarin *et al.*, 2001), which possibly increased the strength of the fruit as a sink for xylemic sap uptake under limited xylem conductivity conditions. The present results showed that fruit xylem sap uptake decreased from 15 to 30 DAP in tomato. Previous studies have shown that phloem may represent 76–83% and xylem may represent 17–24% of fruit peduncle water uptake at early stages of growth and development (Tables 3, 4; De Freitas *et al.*, 2011b). Consistent with the data presented, other studies have also shown that at later stages of growth and development, the xylem contribution to fruit water uptake decreases due to loss of xylem functionality and/or reduction in the hydrostatic gradient responsible for xylemic sap uptake and movement in the fruit (Ho *et al.*, 1993; Dražeta *et al.*, 2004; Bondada *et al.*, 2005; De Freitas *et al.*, 2011b). However, other studies have shown that xylem transport into trusses of tomato fruit cultivar Gourmet remained functional throughout the first 8 weeks of growth. In addition, these studies showed that ~75% of water net influx into the fruit occurred through the external xylem and ~25% via the perimedullary region, which contains both phloem and xylem (Windt *et al.* 2009). Differences in the phloem/xylem ratio of fruit sap uptake presented in the literature could be attributed to different genotypes and/or the growing conditions of each study. In future studies, direct measurements of phloem sap uptake into the fruit using nuclear magnetic resonance should be carried out for the same tomato cultivar and growing conditions as used in the present study to compare precisely the methods and the results obtained (Windt *et al.* 2009).

Although no statistically significant changes in  $\text{Ca}^{2+}$  concentrations in stem xylem sap were observed among the treatments, spraying plants with ABA increased the  $\text{Ca}^{2+}$  concentration in the xylem sap moving into the fruit. The movement of  $\text{Ca}^{2+}$  in the xylem vessels depends on adsorption and desorption of  $\text{Ca}^{2+}$  from active exchange sites within the cell walls (McLaughlin and Wimmer, 1999; Taylor and Locascio, 2004). In that case, fruit of ABA-sprayed plants possibly had exchange sites within the xylem cell walls that were more saturated with  $\text{Ca}^{2+}$ , maintaining higher levels of soluble  $\text{Ca}^{2+}$  in the xylem sap stream. In addition, evidence suggests that special nutrient transport systems exist at the interface between living cells and xylem vessels (De Boer and Volkov, 2003). The higher  $\text{Ca}^{2+}$  concentration observed in the xylem sap of the peduncle of fruit from ABA-sprayed plants could be the result of the higher flow rate of xylem sap into the fruit leading to a higher saturation of  $\text{Ca}^{2+}$  binding sites in the xylem vessels and cell uptake requirements that reduced  $\text{Ca}^{2+}$  binding to active exchange sites in the cell walls as well as the  $\text{Ca}^{2+}$  uptake into living cells at the interface with the xylem vessels.

#### *Calcium concentration in the fruit in response to ABA*

Spraying tomato plants with ABA increased the  $\text{Ca}^{2+}$  concentration and  $\text{Ca}^{2+}$  accumulation in the pericarp tissue at the fruit peduncle end by increasing fruit xylem sap uptake, decreasing fruit phloem sap uptake, increasing  $\text{Ca}^{2+}$  concentration in the xylem sap moving into the fruit, and possibly by increasing phloem  $\text{Ca}^{2+}$  transport into the fruit.

The results show that ABA spray treatment increased fruit xylem sap uptake 4.72-fold, fruit xylem sap  $\text{Ca}^{2+}$  concentration 1.28-fold, and fruit growth 1.41-fold, compared with water spray treatment, respectively. These results suggest that the increase in fruit xylem sap uptake was the most important effect of ABA spray treatment leading to the observed higher fruit  $\text{Ca}^{2+}$  accumulation from 15 to 30 DAP. The  $\text{Ca}^{2+}$  accumulation in fruit tissue estimated by multiplying the xylem sap  $\text{Ca}^{2+}$  concentration in the fruit peduncle by its respective flow rate into the fruit from 15 to 30 DAP was ~84% (average among treatments) of the  $\text{Ca}^{2+}$  accumulation quantified by the difference in total fruit  $\text{Ca}^{2+}$  content observed at 30 DAP minus the total fruit  $\text{Ca}^{2+}$  content observed at 15 DAP. Considering that fruit water uptake is via the xylem and phloem, the results suggest that the phloem may have also contributed to fruit  $\text{Ca}^{2+}$  uptake under the experimental conditions described in this study. The results also show a greater difference between the quantified and estimated  $\text{Ca}^{2+}$  accumulation in the fruit of ABA-sprayed plants ( $6.7 \mu\text{g Ca}^{2+} \text{ fruit}^{-1} \text{ d}^{-1}$ ) than in the fruit of other treatments ( $1.2\text{--}1.5 \mu\text{g Ca}^{2+} \text{ fruit}^{-1} \text{ d}^{-1}$ ), suggesting that spraying plants with ABA also enhanced fruit phloem  $\text{Ca}^{2+}$  uptake. Considering that spraying tomato plants with ABA decreased fruit phloem sap uptake, it is possible that this treatment increased  $\text{Ca}^{2+}$  concentration in the phloem sap to increase fruit  $\text{Ca}^{2+}$  uptake to compensate for the reduction of phloem sap uptake. These results agree with previous studies suggesting that phloem can also have an important contribution to fruit  $\text{Ca}^{2+}$  uptake depending on the phloem sap  $\text{Ca}^{2+}$  concentration and phloem

sap flow rate into the fruit (Jones *et al.*, 1983, 1986; Jones and Samuelson, 1983; De Freitas *et al.*, 2011b). In the present study, it was assumed that fruit transpiration rates were similar among all treatments. Future studies related to the effect of ABA on xylem and phloem fruit water uptake should include direct measurements of fruit transpiration rates.

In the xylem vessels, after reaching the peduncle end of the fruit,  $\text{Ca}^{2+}$  can be taken up by the cells, bind to active exchange sites within the cell walls, or remain soluble in the xylem vessels to be translocated towards the blossom-end tissues of the fruit (McLaughlin and Wimmer, 1999; De Boer and Volkov, 2003; Taylor and Locascio, 2004). Accordingly, the present results show that higher xylem sap and tissue  $\text{Ca}^{2+}$  content at the fruit peduncle end resulted in higher fruit  $\text{Ca}^{2+}$  translocation to and  $\text{Ca}^{2+}$  accumulation in the blossom-end tissues in response to whole-plant ABA treatment.

Dipping the fruit in ABA did not affect xylem sap or tissue  $\text{Ca}^{2+}$  content at the fruit peduncle end, but resulted in higher  $\text{Ca}^{2+}$  accumulation and higher  $\text{Ca}^{2+}$  in the apoplast in the blossom-end tissue at 15 DAP, suggesting that ABA also triggered a fruit-specific mechanism that favoured  $\text{Ca}^{2+}$  translocation from the peduncle end towards the blossom-end region of the fruit. This latter effect was not observed at 30 DAP. According to the present data, spraying the whole plant with ABA or dipping the fruit in ABA maintained a higher number of functional xylem vessels that reduced the resistance to xylemic water and  $\text{Ca}^{2+}$  movement into the blossom-end tissue, which could help to explain the observed higher  $\text{Ca}^{2+}$  content in the distal end of the fruit. In ABA-dipped fruit, the increase in  $\text{Ca}^{2+}$  concentration in the blossom-end tissue was only observed at 15 DAP, possibly due to the reduction in any ABA effect on maintaining a higher number of functional xylem vessels at late stages of fruit growth and development. It is possible that ABA could also increase the number of functional xylem vessels connecting the fruit to the plant, which should be determined in future studies. In addition, higher cuticular wax content in epidermal cells at 30 DAP compared with fruit at 15 DAP (Leide *et al.*, 2007) could limit fruit ABA uptake during the later dip treatments.

#### *Possible mechanisms controlling fruit susceptibility to BER at the whole-plant and fruit-specific levels in response to ABA*

At the whole-plant level, ABA treatment triggered stomatal closure, decreasing xylemic water and  $\text{Ca}^{2+}$  flow to the leaves, which maintained higher stem water potential. Under such conditions, whole-plant ABA treatment favoured xylemic water and  $\text{Ca}^{2+}$  movement into the rapidly expanding fruit, resulting in higher  $\text{Ca}^{2+}$  content reaching the fruit peduncle end. However, the data suggest that xylem sap uptake could not fully explain fruit  $\text{Ca}^{2+}$  accumulation due to the difference between the observed total fruit  $\text{Ca}^{2+}$  accumulation and the estimated fruit  $\text{Ca}^{2+}$  accumulation based on the  $\text{Ca}^{2+}$  concentration in the xylem sap and xylem sap flow rate into the fruit. These results suggest that phloem could have acted as a source of  $\text{Ca}^{2+}$  to the fruit under the experimental conditions described in this study. More detailed studies should

include direct measurements of fruit transpiration rates to better characterize the role of phloem in fruit  $\text{Ca}^{2+}$  uptake. In addition, a better understanding of phloem contributions to fruit  $\text{Ca}^{2+}$  uptake can be accomplished by developing efficient methods to extract and quantify  $\text{Ca}^{2+}$  in the phloem sap moving into the fruit.

At the fruit-specific level, ABA maintained a higher number of functional xylem vessels at early stages of growth and development, reducing the resistance to  $\text{Ca}^{2+}$  movement in the fruit and thus allowing  $\text{Ca}^{2+}$  to be translocated towards the blossom-end tissue. Such  $\text{Ca}^{2+}$  accumulation in the blossom-end tissue was enhanced in response to whole-plant ABA treatment, compared with fruit-specific ABA treatment, possibly due to the additional effects on increasing xylem sap flow rate into the fruit, and  $\text{Ca}^{2+}$  concentration in the xylem sap taken up by the fruit.

Blossom-end tissue has the lowest concentration of  $\text{Ca}^{2+}$  in the fruit, and for that reason is the most susceptible tissue in the fruit to  $\text{Ca}^{2+}$  deficiency disorders (Taylor and Locascio, 2004; Ho and White, 2005). Studies suggest that  $\text{Ca}^{2+}$  deficiency symptoms are triggered by a depletion of the apoplastic pool of  $\text{Ca}^{2+}$  required to bind to phospholipids and proteins on the plasma membrane (White and Broadley, 2003; Taylor and Locascio, 2004; Ho and White, 2005). Under conditions of low apoplastic  $\text{Ca}^{2+}$ , the plasma membrane can become leaky, leading to cell plasmolysis and eventually death (Suzuki *et al.*, 2003; De Freitas *et al.*, 2011a). Accordingly, the results show that higher  $\text{Ca}^{2+}$  accumulation in the blossom-end tissue in response to ABA treatment resulted in higher water-soluble apoplastic  $\text{Ca}^{2+}$  concentration, lower membrane leakage, and reduced fruit susceptibility to BER development. In this context, new tomato cultivars with higher ABA biosynthesis could be selected not only for water saving purposes, but also for reduced fruit susceptibility to BER. However, it is possible that fruit water content will be higher in high ABA genotypes, which may reduced the post-harvest life of fresh fruit.

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